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membrane-associated FtsZ polymers generate a mechanical force for membrane constriction. However, some FtsZ GTPase mutants are viable at permissive temperatures, raising the question of what the role of FtsZ's GTPase activity is in cell division. Previously, fluorescence recovery after photobleaching (FRAP) experiments showed the Z-ring is highly dynamic, and it constantly turns over with cytoplasm FtsZ pool[1]. In this work we used total internal reflection fluorescence microscope (TIRFM) to monitor the dynamics of FtsZ-GFP labelled Z-ring in live E coli cells. We observed that matured Z-rings exhibit highly dynamic oscillatory behaviours in live wild type background cells. These dynamic oscillations are larger in magnitude than the constant individual subunit turnover reported by FRAP. It is also distinct from the previously reported FtsZ oscillation along the cell length of newborn cells before Z-ring's assembly. We further investigated cellular factors and FtsZ properties that could influence this dynamic behaviour.

Reference
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1614-Pos Board B565
Counting Molecules in Non-Muscle Myosin II Filaments
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Non-muscle (NM) myosin II is involved in many important cellular processes, including migration, adhesion and cytokinesis. NM myosin II assembles into bipolar filaments; this multimerization is thought to be essential for actomyosin contractility. Since each head in a myosin filament can bind F-actin, the number of heads per filament will affect the balance among its bundling, crosslinking and sliding activities, as well as the load-dependent feedback on these activities. Electron microscopy studies of interphase cells from different species have shown NM myosin II filaments contain 16-56 heads, but the size of filaments has never been determined in cytokinesis.
We first studied the number of NM-myosin II in meiotic and mitotic C. elegans zygotes. GFP tagged myosin was imaged by TIRF microscopy until the fluorescence signal was completely bleached. We developed a novel, yet very simple molecule counting method CoMPaS (counting molecules with photobleaching and subtraction) to measure the number of bleaching events in the fluorescence decay curves from cells with different level of GFP expression. We found that the total number of myosin molecules in a single filament is 100 - 150. As an independent assessment of myosin filament size, we also measured filament length in cultured Drosophila cells using super-resolution microscopy. We measured the distance between diffraction-limited foci containing GFP-tagged regulatory light chain (RLC) at the two ends of myosin filaments, with nm accuracy. On average, myosin filaments are approximately 380 nm long. This method also allowed us to determine the orientation and size of the RLC myosin head foci. Interestingly, increased myosin filament length correlated with more parallel filament head foci. We will combine these measurement techniques with genetic perturbations to understand how NM myosin II filament assembly is controlled and how interaction with actin influences myosin filament structure.

1615-Pos Board B566
Z-Profiling of CFTR Oligomerization State Distributions via Single Molecule Step Photobleaching Analysis in Epithelial Cells
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The cystic fibrosis transmembrane conductance regulator (CFTR) is an anion channel which conducts chloride and bicarbonate ions through the apical plasma membrane of epithelial cells. CFTR interacts with other proteins which form a macromolecular complex that regulates its activity at the cell surface. The number of CFTR molecules within these complexes remains uncertain due to cell variability and the difficulty of measuring this parameter in situ. We show a single molecule step photobleaching method which measures the oligomerization state of resolved clusters and then maps the z-position profile of individual CFTR molecules within the cluster based on imaging data collected by TIRF microscopy. DNA nanorods with defined fluorescent label spacing were used as controls to validate the analysis and to recover the oligomerization distribution that would be expected for a 24.5 nm building block length (24.6 ± 2.5 nm, measured), and for the 13.5 nm height of the base layer of streptavidin on the coverslip (12.9 ± 1.7 nm). Studies of primary airway epithelial cells expressing GFP-CFTR indicate that CFTR clustering state increases linearly with the z-position from the inside to the outside of the cell. Plots of z position versus oligomer subunit count yielded the same slope under control conditions (6.0 ± 1.4 nm/count) and during protein kinase C stimulation (PKC; 6.0 ± 3.7 nm/count). However, the number of counts per cluster was larger in PKC stimulated cells. Our results demonstrate that CFTR aggregation state varies with depth from the cell surface, and may be related to the mechanism of cluster assembly inside the cell and/or its function at the plasma membrane. We anticipate that our method can be generalized to monitor the z-position profile or oligomerization states of other membrane receptors and channel to determine structure/function relationships for such membrane complexes.

1616-Pos Board B567
Dimerization of EphA2 in Cell Membranes
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EphA2 is a single pass transmembrane protein and a member of the largest Receptor Tyrosine Kinase (RTK) family. EphA2 regulates cell-cell interactions during embryonic development in humans and is known as an oncoprotein as well as a tumor suppressor. The Eph receptors differ from other RTKs since they form oligomers when they bind their ligands. However, the interactions between the EphA2 receptors in the absence of ligand have not been investigated. We used spectral FRET in conjunction with two photon microscopy to probe unliganded EphA2 dimerization in HEK 293 cells. We measured the FRET efficiency with high precision over a concentration range that spans three orders of magnitude and includes the physiological range of receptor expressions. We show that EphA2 forms dimers in the absence of ligand. In addition, mutagenesis studies reveal that contacts between the EphA2 receptors that are important for ligand-mediated clustering are also important for unliganded dimerization. We therefore propose that unliganded EphA2 dimers are an important intermediate in EphA2 signal transduction.

1617-Pos Board B568
Single Molecule Analysis Reveals Coexistence of Stable Serotonin Transporter Monomers and Oligomers in the Live Cell Plasma Membrane
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The human serotonin transporter (hSERT) is responsible for the termination of synaptic serotonergic signaling. The formation of SERT oligomers in the plasma membrane has already been indicated by various approaches, including optical methods such as Förster resonance energy transfer (FRET) and classical biochemical ensemble analysis. However, neither application is suitable to yield quantitative interpretation and decipher the exact configuration of the oligomerization states; additionally, biochemical methods do not take the influence of the membrane environment into account. Here we used single molecule fluorescence microscopy to obtain the oligomerization state of SERT via brightness analysis of single diffraction limited fluorescent spots. The techniques applied in this study allow for identification and quantitative evaluation of subpopulations of SERT complexes exhibiting different degrees of oligomerization in a living cell. We found a variety of oligomerization states of membrane-associated transporters, revealing molecular associations at least up to pentamers and demonstrating the coexistence of different degrees of oligomerization in a single cell. The oligomerization was found to be independent of SERT surface density, and the interactions were stable over several minutes. Together, these results indicate kinetic trapping of preformed SERT oligomers at the plasma membrane. Next, we developed a strategy for single molecule analysis at the membrane of the endoplasmic reticulum. By evaluating the oligomerization of SERT in the ER we found that the oligomerization process is chemically equilibrated at ER membranes; after trafficking to the plasma membrane, the SERT stoichiometry remains fixed.
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1618-Pos Board B569
RFP Tags for Labeling Secretory Pathway Proteins
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Many RFPs are prone to form artificial puncta when labeling proteins in secretory pathway, which may severely impede their further uses in living cell